

SANZ MOLINERO  
Appl. No. 10/553,656  
Atty. Ref.: 4982-13  
April 8, 2008  
Amendment

**AMENDMENTS TO THE DRAWINGS**

Please insert the attached two (2) Replacement Sheets of drawings in place of the drawings filed October 14, 2005. Also attached are four (4) Annotated Sheets of drawings showing the changes in the drawings to cancel Figure 4 (2 sheets). No new matter has been added.

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### **REMARKS**

Reconsideration is requested.

Claims 8 and 11-24 have been canceled, without prejudice.

Claims 1-7, 9, 10 and 25-34 are pending. Claims 25-34 have been added and find support in, for example, originally-filed claims 1-10. No new matter has been added.

Copies of the previously-cited documents are attached along with a further PTO 1449 Form citing same as well as additionally attached documents. Return of an initialed copy of the PTO 1449 Form filed October 14, 2005, pursuant to MPEP § 609, is requested.

The Examiner is requested to provide a complete PTO 892 Form, which includes the title of each cited Non-Patent Document.

Specifically, the PTO 892 Form received with the Office Action of October 18, 2007 fails to include the title of each Non-Patent Document.

The Examiner will appreciate that MPEP § 707.05(e) provides as follows:

#### **707.05(e) Data Used in Citing References [R-2]**

37 CFR 1.104(d) (see also MPEP § 707.05 and § 901.05(a)) requires the examiner to provide certain data when citing references. The examiner should provide the citations on the "Notice of References Cited" form PTO-892 (copy at MPEP § 707.05). ...

#### **III. < PUBLICATIONS**

In citing a publication, sufficient information should be given to determine the identity and facilitate the location of the publication. ...

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In citing periodicals, information sufficient to identify the article includes the author(s) and title of the article and the title, volume number issue number, date, and pages of the periodical.

See  
[http://www.uspto.gov/web/offices/pac/mpep/documents/0700\\_707\\_05\\_e.htm#sect707.05e](http://www.uspto.gov/web/offices/pac/mpep/documents/0700_707_05_e.htm#sect707.05e) (August 29, 2007) (Emphasis added.)

The Examiner is requested to provide a new PTO 892 Form which includes the information required by the MPEP, such as is described in the above-quoted passage.

The specification has been amended above to include the attached Sequence Listing. The attached paper and computer readable copies of the Sequence Listing are the same. No new matter has been added.

The specification has been amended to include sequence identifiers in the description of Figure 1 and elsewhere as appropriate. No new matter has been added.

The specification has been amended to delete browser executable text. No new matter has been added.

The drawings have been amended to insert the attached two (2) Replacement Sheets of drawings in place of the drawings filed October 14, 2005. Also attached are four (4) Annotated Sheets of drawings showing the changes in the drawings to cancel Figure 4 (2 sheets). No new matter has been added.

Withdrawal of the objections to the specification and drawings is requested.

The Section 112, second paragraph, rejection of claims 1-10 is obviated by the above amendments. Withdrawal of the rejection is requested.

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The Section 112, second paragraph, rejection of claims 1 and 4 stated on page 6 of the Office Action dated October 18, 2007 is obviated by the above amendment. Withdrawal of the rejection is requested.

The Section 112, second paragraph, rejection of claims 2, 7 and 9 stated on page 7 of the Office Action dated October 18, 2007 is obviated by the above amendments. Withdrawal of the rejection is requested.

To the extent not obviated by the above amendments, the Section 112, first paragraph "enablement", rejection of claims 1-10 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following comments.

The Examiner's indication that the specification is enabling for a method of increasing plant seed yield comprising transformation of a plant with a nucleic acid sequence encoding a metallothionein protein as defined in SEQ ID NO:2, is acknowledged with appreciation. See page 7 of the Office Action dated October 18, 2007.

The claims have been amended, without prejudice, to advance prosecution. The applicants submit that metallothionein proteins are well known in the art, such as is evidenced by Robinson et al (Biochem J. 295; 1-10, 1993) cited by the Examiner. Specifically, for example, the applicants believe the reference states on page 1, left column, line 24-26 that a wealth of information is available concerning the structure of animal and fungal metallothioneins. Cobbet and Goldsbrough (Annu. Rev. Plant Biol. 53, 159-1 82, 2002; cited in the present specification) state on page 170, lines 20-21

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that the number of characterized plant metallothionein proteins has increased dramatically. On the same page, lines 25-26 they state that plant type 2 metallothioneins follow the classification of Robinson et al. This implies that the information available for animal and fungal metallothioneins of type 2 can be extrapolated to plant type 2 metallothioneins. There is ample information on metallothioneins available in the prior art such that the ordinarily skilled person given the present specification will be able to make and use the claimed invention without undue experimentation, The documents cited on page 10 of the Office Action dated October 18, 2007 do not relate to metallothionein proteins and cannot be extrapolated as such to metallothionein proteins. The Examiner is requested to clarify the relevance of the documents cited on page 10 of the Office Action to the presently claimed invention, especially in view of the apparent wealth of information regarding specific proteins more directly related to the claimed invention.

As for the Examiner's comments on page 11 of the Office Action dated October 18, 2007, the applicants submit that the Examiner's assertions appear to be based on the function of metallothioneins under natural conditions, which cannot be compared to the presently claimed invention, where metallothionein proteins are expressed under artificial conditions. As a result of the claimed method, the metallothionein proteins is expressed in cells or tissues, or during developmental stages, where it is not normally expressed. Therefore the applicants believe that the function of metallothioneins under natural conditions is not comparable to the function of metallothioneins expressed under

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artificial conditions and that no extrapolations should be made, as appears to be asserted by the Examiner.

The claims are submitted to be supported by an enabling disclosure. The applicants submit that one of ordinary skill in the art will be able to make and use the claimed invention without undue experimentation. Withdrawal of the Section 112, first paragraph "enablement", rejection is requested.

To the extent not obviated by the above amendments, the Section 112, first paragraph "written description", rejection of claims 1-10 is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the disclosure on page 8, lines 5-9 of the specification, for example, Withdrawal of the "written description" rejection is requested.

The Section 102 rejection of claims 1-5 and 8-10 over Basel (WO 98/36084), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

Basel et al. does not disclose the metallothionein of the claimed invention, such as is represented by SEQ ID NO: 2. Rather, the mentioned metallothionein protein in WO 98/36084 (SEQ ID NO: 7) is a partial (see page 23 of the cited document, lines 4-9, in which paragraph the function is specified as metal binding) and has only 11.2 % sequence identity to SEQ ID NO: 2 of the present invention. Further, the cited document fails to describe the conserved sequence, i.e., SEQ ID NO: 9, of the present invention. WO 98/36084 fails to teach each and every aspect of the claimed invention .

Furthermore, the disclosure by Basel states that the improved growth rates with postulated yield increase is obtained upon combined expression of calcium and metal binding genes (page 9, lines 1-5), wherein the metallothionein only increases growth rate (page 9, lines 7-17; page 36, line 36 to page 37, line 5). The cited document fails to show that metallothionein expression (either alone or in combination with other genes) results in increased plant yield.

The Examiner's apparent belief that increased yield would be inherent to faster plant growth rates has not been supported by citation to any technical literature for example. There is no established link between faster growth rates and increases in yield. The applicants note, for example, that He et al. (Transgenic Research 9, 223-227, 2000) describe rice plants transformed with LEAFY that showed a faster growth rate but had no yield increase. On the contrary both biomass and the seed yield were reduced (abstract and Table 1 on page 226). Jeon et al. (Molecular Breeding 6, 581-592, 2000) describe similar observations with rice plants ectopically expressing MADS-box genes (see pages 584 to 587 with description of the phenotypes), the plants were dwarfed (reduced biomass yield) and reduced fertility (reduced seed yield).

The claims are submitted to be patentable over Basel (WO 98/36084) and withdrawal of the Section 102 rejection based on the same is requested.

The Section 103 rejection of claims 1-10 over Basel (WO 98/36084) in view of Zhou (Mol. Gen. Genetics 248:318-328, 1995), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the above and the following distinguishing comments.

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The secondary reference fails to cure the deficiencies of Basel noted above.

Specifically, Basel et al does not teach a method of making a transgenic plant with increased growth and development, as asserted by the Examiner . Basel et al. teach, at best, that plants expressing a partial metallothionein protein results in increased growth rate.

Zhou et al. teach that metallothionein proteins play a role in metal uptake and in tolerance to heavy metals. This property of tolerance to heavy metals is also used by Basel et al., where the expression of the metallothionein protein fragment was used as a selection tool (see for example page 72, line 16-26).

However neither Zhou et al. nor Basel et al. suggest that ectopically expressing in a plant a metallothionein gene as such would result in increased yield. The expression pattern of MT2 described in Zhou does not provide any suggestion as to the effect of MT2 on plant yield, for example, expression in the inflorescence may affect development of flowers but is not predictive for the development of seeds.

The claims are submitted to be patentable over the combination of Basel and Zhou and withdrawal of the Section 103 rejection is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned, preferably by telephone, in the event anything further is required to place the application in condition for allowance.



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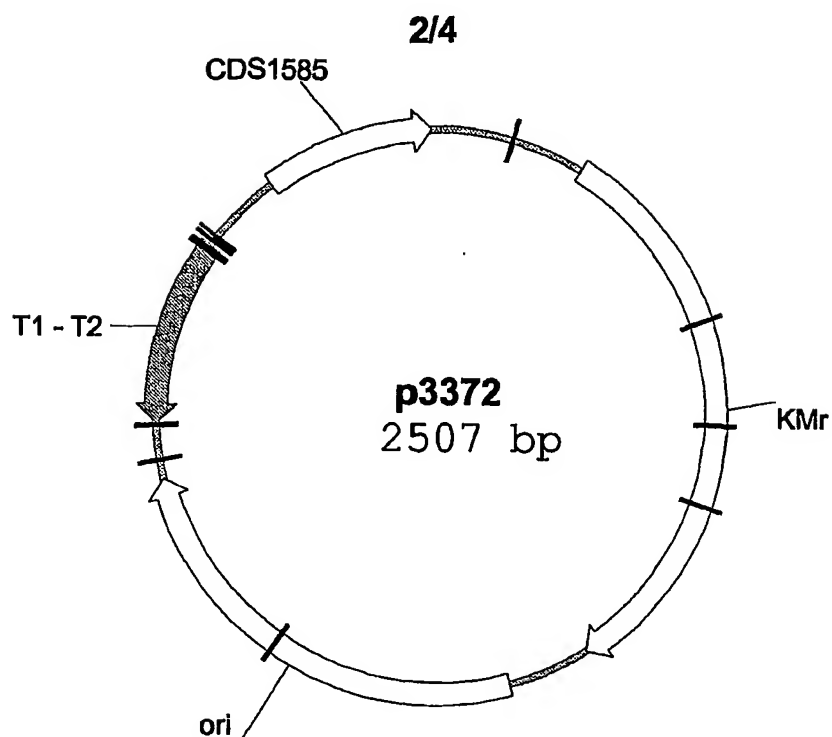
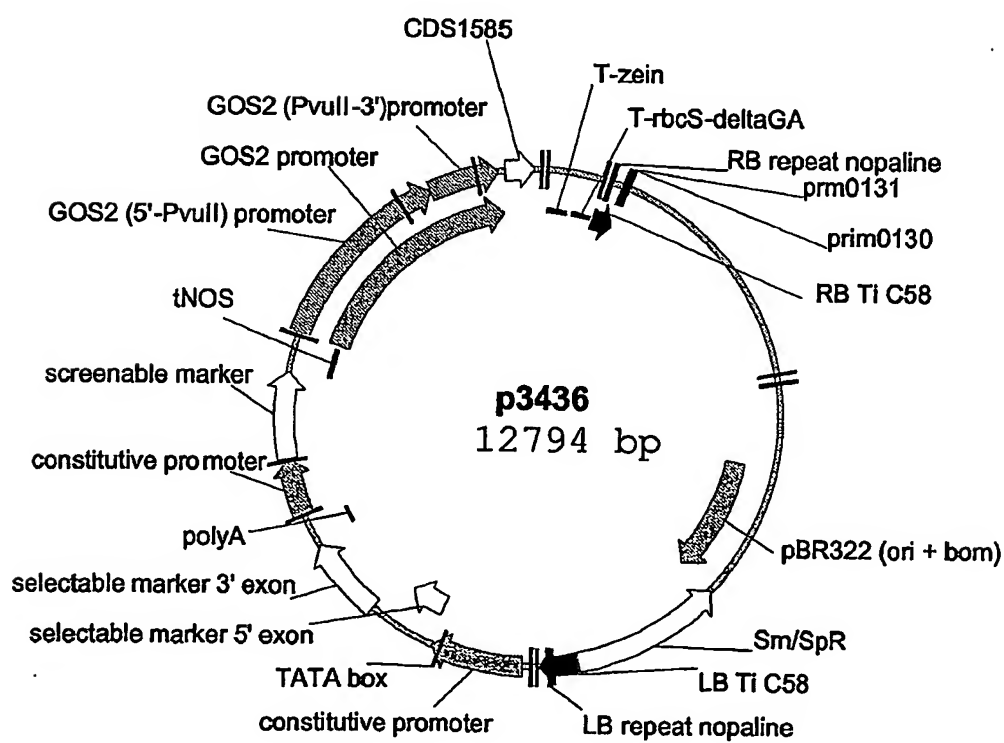
Respectfully submitted,

**NIXON & VANDERHYE P.C.**

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                    Reg. No. 36,663

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Facsimile: (703) 816-4100



**FIGURE 2****FIGURE 3**

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**SEQ ID NO 1: AtMT2a (CDS1585) coding sequence (start and stop in bold)**

ttttcattcataaattttttcttcaatttgaattttctcgagaaaaat**gt**ccttgctgtggagg  
aaactgcggatgtggatctggctgcaagtgcggcaacggttgtggagggttgcaaaatgtacc  
ctgacttgggattctccggcgagacaaccacaactgagacttttgtcttgggcgttgcaccg  
gcatgaagaatcagtacgaggcttcaggggagagtaacaacgctgagaacgatgcttgcaa  
gtgtggatctgactgcaagtgtgatccttgacctgcaagt**ga**agaagcctttttaataag  
cagagataatcgagtctctttaatta

**SEQ ID NO 2: AtMT2a (CDS1585) deduced protein sequence**

MSCCGGNCGCGSGCKCGNGCGGCKMYPDLGFSGETTTTETFLGVAPAMKNQYEASGESNNA  
ENDACKCGSDCKCDPCTCK

**SEQ ID NO 3: A. thaliana AtMT-1 cDNA, start and stop codon in bold.**

cattcataaattttttcttcaatttgaattttctcgagaaaaat**gt**ccttgctgtggaggaaac  
tgcgatgtggatctggctgcaagtgcggcaacggttgtggagggttgcaaaatgtaccctga  
cttgggattctccggcgagacaaccacaactgagacttttgtcttgggcgttgcaccggcga  
tgaagaatcagtacgaggcttcaggggagagtaacaacgctgagagcgatgcttgcaagtgt  
ggatctgactgcaagtgtgatccttgacctgcaagt**ga**agaagcctttttaataagcaga  
gataatcgagtctctttaatntaattaagtattcaataagtaaaccatatataggatgggtg  
tttttaggttttggtttatgtgtaataatggcttcagcttatcttttagccgatcattgtctt  
ttgtgtttgtttgatcataatcttttagatgtctagcaaatctgccatgtgatgagtttgta  
cttcagtggaatgataataatattatagttttaaatcaaaaaaaaaa

**SEQ ID NO 4: A. thaliana AtMT-1 deduced protein sequence**

MSCCGGNCGCGSGCKCGNGCGGCKMYPDLGFSGETTTTETFLGVAPAMKNQYEASGESNNA  
ESDACKCGSDCKCDPCTCK

**SEQ ID NO 5: prm03240**

GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACAATGTCTTGCTGTGGAGGAA

**SEQ ID NO 6: prm03241**

GGGGACCACTTTGTACAAGAAAGCTGGGTTTCACTTGCAGGTGCAAG

**SEQ ID NO 7: Expression cassette for MT2a**

aatccgaaaagtttctgcaccgttttcccccctaactaacaatatagggaacgtgtgctaa  
atataaaatgagaccttatatatgtagcgctgataactagaactatgcaagaaaaactcatc  
cacctacttttagtggcaatcgggctaataaaaaaagagtcgctacactagtttcgttttccct

**FIGURE 4**

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tagtaattaagtgggaaaatgaaatcattattgcttagaatatacgttcacatctctgtcat  
gaagttaaattattcgaggtagccataattgtcatcaaactcttcttgaataaaaaaatctt  
tctagctgaactcaatgggtaaagagagagatttttttaaaaaatagaatgaagatattc  
tgaacgtattggcaaagatttaaacatataattatataattttatagtttgtcattcgtca  
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tgtgctcatgtgcatgtgtgagtgcacctcctcaatacacgttcaactagcaacacatctct  
aatatcactcgcctatttaatacatttaggttagcaatatctgaattcaagcactccaccatc  
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tgtgatgattcctgttcttggtttgggtagaggggttcttgatgttgcatgttatcggtt  
cggtttgatttagtagtatgggttttcaatcgtctggagagctctatggaaatgaaatggttta  
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attttgcttggtgtaataaaagtacgggttgtttgggtcctcgattctggtagtgatgcttctc  
gatttgacgaagctatcctttgtttattccctattgaacaaaaataatccaactttgaagac  
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gtttagatacagtagtccccatcacgaaattcatggaaacagttataatcctcagggaacagg  
ggattccctgttcttccgatttgctttagtcccagaattttttttcccaatatcttaaaaa  
gtcactttctgggttcagttcaatgaattgattgctacaaataatgcttttatagcgttatcc  
tagctgtagttcagttaataggtaataccctctatagtttagtcaggagaagaacttatccga  
tttctgatctccatttttaattatataatgaatgaactgtagcataagcagttattcatttgat  
tattttttttattagctctcacccttcattattctgagctgaaagtctggcatgaactgtc  
ctcaattttgttttcaaattcacatcgattatctatgcattatcctcttgatctacctgta  
gaagtctcttttgggttattccttgactgcttgattacagaaagaaatttatgaagctgtaa  
tcgggtagattatactgcttgttcttatgattcatttcccttgtgcagttcttgggtgtagct  
tgccactttcaccagcaaagttcattttaaatcaactagggtatcacaaagtttgtaaaaaa  
agcagggttcacaatgtcttgctgtggaggaaactgcggatgtggatctggctgcaagtgcg  
gcaacgggttggtggaggttgcaaaatgtaccctgacttgggattctccggcgagacaaccaca  
actgagacttttgtcttgggcgttgccacggcgatgaagaatcagtagcagggttcaggga  
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cttttaataggtttatacaataattgatatatgttttctgtatatgtctaatttggttatcat  
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gagaaaaaacaataatattaatttgagaatgaacaaaaggaccatatcattcattaactct  
ctccatccatttccatttcacagttcgatagcgaaaaccgaataaaaaacacagtaaat  
caagcacacaaatggtacaagaaaaacagttttcccaatgccataatactcgaac

FIGURE 4 (continued)